Using phage integrases in a site-specific dual integrase cassette exchange strategy.

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Public Summary:
This chapter provides a detailed protocol for carrying out a useful two-step method for precise genomic modification that was developed in the Calos lab. In this method, a "landing pad" DNA sequence is placed into the genome of human induced pluripotent stem cells by using homologous recombination, a natural process that occurs in most cells. Since homologous recombination is not a frequent event, the frequency was stimulated by using a pair of TALENs, which are nucleases that can cut DNA at a specific DNA sequence. This cut acts to stimulate recombination at this location in the genome. The location in the genome that we targeted was a region called H11 that is found on human chromosome 22. H11 is an excellent place to position an incoming gene, because gene addition at H11 does not disrupt other genes and does not lead to changes that could cause cancer. Furthermore, genes placed into this location are expressed long-term in a wide variety of cell types. The landing pad that we positioned at H11 carried the neomycin resistance gene and the gene for green fluorescent protein. These genes make it easy to find cells that have undergone recombination and inserted the landing pad. These marker genes were flanked by the recognition sites for the phiC31 and Bxb1 integrase enzymes. When a colony of cells carrying the correctly-inserted landing pad was identified by producing the expected fragments in a polymerase chain reaction analysis, step one was complete. In step two, any gene that is desired can be inserted into the landing pad. The desired gene is flanked by recognition sites for the phiC31 and Bxb1 integrases, and is co-introduced into the cells, along with plasmid DNA encoding the two integrases. The genes for puromycin resistance and mCherry are also included, for easy identification of the desired insertion event. Thus, we were able to select clones that had successfully undergone the dual integrate cassette exchange reaction by choosing puromycin-resistant colonies that were mCherry-positive and GFP-negative. This method has a high efficiency and specificity and allows for precise identification of correctly integrated clones. We provide in this chapter the detailed steps for carrying out this method, which is useful for precise modification of stem cells.

Scientific Abstract:
PhiC31 integrase, a site-specific large serine recombinase, is a useful tool for genome engineering in a variety of eukaryotic species and cell types. PhiC31 integrase performs efficient recombination between its attB site and either its own placed attP site or a partially mismatched genomic pseudo attP site. Bxb1 integrase, another large serine recombinase, has a similar level of recombinational activity, but recognizes only its own attB and attP sites. Previously, we have used these integrases sequentially to integrate plasmid DNA into the genome. This approach relied on placing a landing pad attP for Bxb1 integrase in the genome by using phiC31 integrase-mediated recombination at a genomic pseudo attP site. In this chapter, we present a protocol for using these integrases simultaneously to facilitate cassette exchange at a predefined location. This approach permits greater control and accuracy over integration. We also present a general method for using polymerase chain reaction assays to verify that the desired cassette exchange occurred successfully.